

Effect of guinea fowl egg storage duration on embryonic and physiological parameters, and keet juvenile growth

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ABSTRACT This study investigated the effects of guinea fowl hatching eggs storage time on embryo parameters and post-hatch juvenile growth. A total 1,800 eggs of guinea fowl were used. They were numbered, weighed, and divided into four groups of 450 eggs each according to storage time of 3, 7, 11, and 15 D before storage at a temperature of 18°C. Then, they were incubated at 37.7°C and 55% relative humidity for 28 D in a forced-draft incubator. Egg weight loss, albumen pH and weight, embryo weight, hatching events, and keet growth up to 7 D post-hatch were recorded. In addition, thyroid hormone and corticosterone levels were determined. The results indicate that during storage, relative egg weight loss increased with storage duration. However, albumen pH increased with storage time up to 11 D of storage and remained unchanged between 11 and 15 D. In addition, from 19 to 22 D of incubation, albumen weight was higher for eggs stored for 15 D compared to that of eggs stored for 3 to

11 D. But, from 16 D of incubation, embryos from eggs stored for 3 D grew faster than those from eggs stored for 7 to 15 D. Incubation durations up to internal pipping (**IP**), external pipping (**EP**), and hatching events increased with egg storage duration. At IP, corticosterone and triiodothyronine (T3) concentrations of eggs stored for 15 D had the lowest ($P < 0.05$) compared to those of eggs stored for 3 to 11 D. Moreover, the levels of thyroxine (T4) decreased with storage duration ($P < 0.05$). At hatch, corticosterone levels increased while T4 levels decreased with storage duration ($P < 0.05$). Also, hatchability decreased with egg storage duration. In addition, 7-day-old keets from eggs stored for 3 and 7 D had comparable weight and were heavier than those from eggs stored for 11 D. It was concluded that storage of guinea fowl hatching eggs more than 7 D negatively affects egg quality and subsequently depresses embryo and post-hatch growth.

Key words: guinea fowl egg, embryo development, hatching parameter, thyroid hormone, corticosterone

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INTRODUCTION

Guinea fowl originates from Africa (Ikani and Dafwang, 2004), where it has a cultural significance (Kone et al., 2018) and its raising is considered as an important traditional activity and food source (Konlan et al., 2011). As a food source, Mareko et al. (2008) reported that guinea fowls had a high carcass yield and Ayorinde (1991) reported high protein and low fat carcass. Konlan et al. (2011) reported that guinea fowl are able to lay throughout the year if they are fed ad libitum with adequate feed. In natural conditions, a guinea fowl can lay up 15 eggs per clutch indicating almost 14 to 20 D between the first and the last egg (Moreki, 2009).

Several studies have shown that egg storage duration before incubation affects eggs quality and hatchability of chickens (Lapaô et al., 1999; Tebesi et al., 2002; Tona et al., 2003; Tilki and Saatchi, 2004; Cağlayan et al., 2009; Dudusola, 2009). Moreover, Cağlayan et al. (2009) pointed out that the yolk-to-albumen ratio increased with storage duration due to evaporative water loss from albumen. Tona et al. (2003) and Moreki and Ditshupo (2012) reported positive correlation between incubation and storage durations. In addition, Fasenko et al. (1992) and Whitehead et al. (2002) pointed out that the optimum storage duration of chicken hatching egg is 7 D and every extension of this duration decreases egg quality, increases embryonic mortality, and extends incubation time. This prolonged incubation time indicates that embryo growth trajectory is delayed by deterioration in egg quality during storage. The change in embryo growth trajectory may be related to changes in embryo physiological parameters. Indeed, it was shown that hatching time affects tri-iodothyronine (T3), thyroxine (T4), and corticosterone concentrations in chicks

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(Tona et al., 2003 and Morita et al., 2016). This observation indicates that the developmental stage is a major determinant of corticosterone, T3, and T4 concentrations. Since these hormones are well known to play critical roles in hatching events in precocial birds as the chicken, it can be hypothesized that the changes in their concentrations in guinea fowl embryos and keets may be linked to hatching eggs storage condition. To our knowledge, most of the studies have focused on chicken hatching egg while guinea fowl has been given very little attention. Therefore, this study aimed to investigate the effects of storage of guinea fowl hatching eggs for 3 to 15 D on embryo parameters, hatching events, levels of corticosterone, T3, T4, and post-hatch juvenile growth.

MATERIALS AND METHODS

Experimental Design

A total of 1,800 hatching eggs produced by Galor G1543 guinea fowl breeders of 32 wk of age and provided by Incubel n.v. (Hoogstraten, Belgium) were studied. They were numbered, weighed, and divided into 4 treatment groups of 450 eggs each. Each group was assigned to different storage duration of 3, 7, 11, and 15 D at 18°C and relative humidity of 70%. Prior to incubation, the eggs from each treatment group were weighed again and divided into 3 replications of 150 eggs each. The eggs were incubated successively in the same incubator at a temperature of 37.7°C, relative humidity of 55%, and turning once an hour until 23 D in Petersime incubator Visio 96. At setting, days 2 and 6 of incubation, samples of 12 eggs per storage time were used to determine thick albumen pH. Moreover, from the 10th to 22nd day of incubation, samples of 15 eggs per storage time were removed from the incubator and broken out every 3 D to weigh embryo and remaining albumen. At the end of 23 D of incubation, the eggs were weighed, candled, and those with evidence of living embryo were transferred into hatching baskets. During the last 4 D of incubation, hatching events such as internal pipping (IP), external pipping (EP), and hatch were monitored every 2 h. At IP stage and at hatch, samples of 30 eggs and 30 keets, respectively, per storage time were used to collect blood sample for the determination of levels of T3, T4, and corticosterone. At the end of incubation, sample of 1-day-old guinea fowl were reared for 7 D and weighed.

Albumen pH Measurement

At 2 and 6 D of incubation, samples of 12 eggs (4 eggs per replication) and according to the storage length were broken out to measure thick albumen pH. For each broken egg, the pH of the thick albumen was measured with a pH meter after calibration of the electrode with buffered solutions of pH 7 and 10. Between 2

consecutive measurements, the probe was cleaned with distilled water. Thick albumen pH was measured with an oxythermometer VWR- pH 110 and the accuracy was 0.01.

Egg, Albumen, Embryo, and Keet Weighing

Egg weights were recorded at setting and at day 23 of incubation, and were used to determine relative egg weight loss during incubation as: $\text{Egg weight loss} = 100 \times (\text{egg weight at setting} - \text{egg weight at day 23 of incubation}) / \text{egg weight at setting}$. Every three days, from 10 to 22 D of incubation, a sample of 15 eggs per treatment were opened to record embryo weights. Also, remaining albumen from the same eggs were weighed.

Hatching Events

At the end of 23 D of incubation, the eggs were candled and those with the evidence of living embryos were transferred into the hatching basket. From 576 to 696 hr of incubation, transferred eggs were checked individually every 2 hr for pipping and hatching events. During this period, times of IP, EP, and emergence for each egg were recorded. These data were used to calculate the duration of IP (time of EP – time of IP), duration of EP (hatching time – EP time), and duration of hatch (hatching time to IP). Total incubation duration was also calculated as the time between setting and hatching. All candled and unhatched eggs were opened to classify them as “infertile” or eggs with dead embryos. Dead embryos were classified as early dead (first 7 D of incubation) and late dead (from 8 D of incubation onwards). The numbers of dead embryos and hatched chicks were used to calculate embryo mortality and hatchability according to fertile eggs and storage treatment as:

$$\text{Mortality} = 100 \times \frac{\text{Number of dead embryos}}{\text{Number of fertile eggs}} \quad (1)$$

$$\text{Hatchability} = 100 \times \frac{\text{Number of hatched chicks}}{\text{Number of fertile eggs}} \quad (2)$$

Post-hatch Juvenile Management

At the end of incubation, 1-day-old keets were grouped according to storage treatment. A sample of 60 keets per treatment (240 keets in total) were weighted and divided into 3 replications of 20 keets each. Within the group, 3 replications were reared for 7 D, distributed randomly in poultry house, in order to avoid positional effects in the facility and on a floor litter with a density of 20 birds per m². During the rearing period, the birds had access to water and feed ad libitum. Birds were weighted individually at the end of 7 D and pen average was calculated.

Table 1. Effect of storage time on egg weight and weight loss during incubation.

Parameters	Storage time (D)			
	3	7	11	15
Egg weight (g)	41.15 ± 4.30 ^a	42.72 ± 3.07 ^a	42.25 ± 2.92 ^a	42.54 ± 3.27 ^a
Weight loss during storage (%)	0.00 ± 0.00 ^c	1.37 ± 0.24 ^b	1.52 ± 0.3 ^b	2.35 ± 0.31 ^a
Weight loss during incubation (%)	11.20 ± 1.10 ^a	11.28 ± 0.37 ^a	11.43 ± 0.64 ^a	11.42 ± 0.65 ^a

^{a-c}Means ± SD within columns with no common superscripts differ significantly ($P < 0.01$).

T3, T4, and Corticosterone Levels Determination

Blood samples were collected from 30 embryos (10/replication) at IP and from 30-day-old keets (10/replication) per treatment for determination of levels of T3, T4, and corticosterone. Blood was collected by cardiac aspiration from embryos and from jugular vein of keet.

The blood samples were further centrifuged at 3,000 rpm for 15 min, and the sera obtained were stored in a freezer at -20°C . A volume of 100 μL of serum was used for corticosterone, T4, and T3 concentrations determination by using the automated VIDAS systems, which is an enzyme-linked fluorescent assay (ELFA) technique. Antibody anti-T3 of mutton, marked by phosphatase alkaline and sodium azide, antibody anti-T4, marked by phosphatase alkaline and methylisothiazolone, and a derivative of cortisol, marked by phosphatase alkaline and sodium azide, provided by VIDAS were used, for the determination of the concentrations of tri-iodothyronine, tyroxine, and corticosterone, respectively. For each hormone, all the samples were run in the same assay in order to avoid inter-assay variability.

Statistical Analysis

The data were processed with the SAS statistical software package (SAS Version 6.124).

One way ANOVA model was used to analyze the albumen pH, egg weight loss, hatching events durations, incubation time, and the weights of remaining albumen and keets. The model was as follows:

$$Y_i = \mu + \alpha_i + \varepsilon_i,$$

where, Y_i = pH, egg and remaining albumen weights, T3, T4, corticosterone, IP and EP duration or hatching time of egg from storage time i , μ = overall mean, α_i = main effect of storage time i , and ε_i = random error term from storage.

Embryonic mortality and hatchability were analyzed using a proc logistic procedure. When the overall F-value was statistically significant ($P < 0.05$), further comparisons among groups were made according to Tukey's test.

RESULTS

Effect of Storage Duration on Egg Weight Loss

Table 1 shows egg weights and relative egg weight losses during storage and incubation. Overall, egg weights varied from 38.43 to 51.26. Between storage groups, egg weights and egg weight loss during the 23 D of incubation were comparable. But, egg weight loss increased with storage duration ($P < 0.01$).

Effect of Storage Duration on pH, Embryo Weight, and Remaining Albumen

Albumen pH before setting eggs for incubation and at 2 and 6 D of incubation according to storage duration are shown in Table 2. Prior to setting the eggs for incubation, albumen pH increased ($P < 0.05$) with storage time up to 11 D of storage and remained comparable between 11 and 15 D of storage. At day 2 of incubation, albumen pH of eggs stored from 7 to 15 D were comparable but higher than those of eggs stored for 3 D ($P < 0.05$). Albumen pH for all storage treatments were similar at day 6 of incubation. Irrespective of storage duration, the lowest albumen pH values were obtained at day 6 of incubation ($P < 0.05$) compared to those of eggs before setting for incubation and at 2 D of incubation that were similar.

As shown in Figure 1, remaining albumen weight decreased with incubation time. Between 10 and 16 D of incubation, remaining albumen weights were comparable between storage treatments. However, from 19 to 22 D of incubation, remaining albumen was higher for eggs stored for 15 D compared to those of eggs stored for 3 to 11 D ($P < 0.001$).

Effect of Storage Time on Embryo Weight and Mortality, Hatchability, and Hatching Events

Figure 2 indicates that embryo weights increased with incubation time in all storage groups. With regard to storage duration, embryo weights from 10 to 16 D were similar between groups. But from day 19 onwards, embryos from eggs stored for 3 D were heavier than those from eggs stored for 7 to 15 D ($P < 0.001$).

Table 2. Cumulative effect of storage duration on albumen pH during the first 6 D of incubation.

Incubation time (D)	Storage time (D)			
	3	7	11	15
0	9.64 ± 0.07 ^c	10.03 ± 0.04 ^b	10.17 ± 0.08 ^a	10.20 ± 0.07 ^a
2	9.70 ± 0.17 ^b	9.99 ± 0.05 ^a	9.99 ± 0.11 ^a	10.01 ± 0.05 ^a
6	8.54 ± 0.62 ^{a*}	8.94 ± 0.26 ^{a*}	8.74 ± 0.28 ^{a*}	8.87 ± 0.30 ^{a*}

^{a-c}Within lines, data sharing no common letter are different and with column * indicates difference between data ($P < 0.05$).

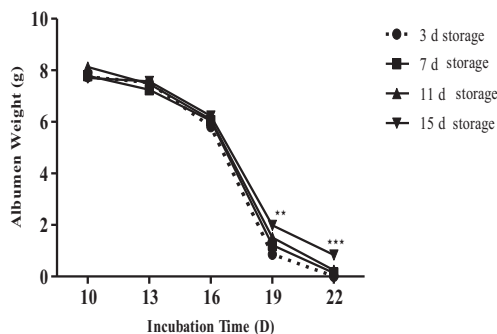
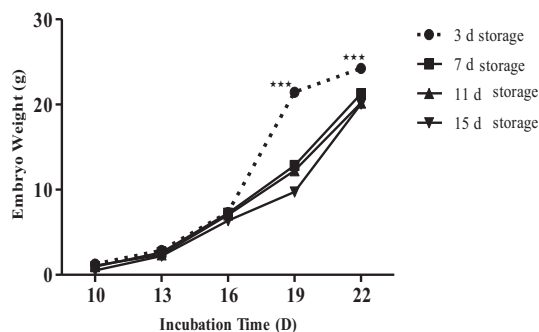
**Figure 1.** Evolution of albumen weights of guinea fowl eggs under different storage duration.**Figure 2.** Embryonic weights of guinea fowl eggs under different storage duration.

Table 3 shows embryo mortality and hatchability according to the storage duration. Overall, early and late embryonic mortalities increased while hatchability decreased rapidly with storage duration ($P < 0.05$). Also, incubation durations up to IP, EP, and hatch as well as durations of hatching events (IP, EP, and hatch) increased with egg storage duration (Table 4) ($P < 0.001$).

Effect of Storage Time on Keets Weights

Weights of guinea fowl keets at hatch and 7 D old are shown in Figure 3. At hatch, the weights were similar between all storage treatment groups. At 7 D of age, keets from eggs stored for 3 and 7 D had comparable body weights and were the heaviest ($P < 0.001$). Body weight of keets from eggs stored for 11 D were higher than those from 15 D storage eggs that had the lowest body weight ($P < 0.01$).

Effects of Storage Duration on T3, T4, and Corticosterone Concentrations

Table 5 shows levels of corticosterone, T3 and T4 at IP and at hatch according to storage durations. At IP, corticosterone and T3 concentrations were comparable for embryo from eggs stored for 3 to 11 D. Embryo from egg stored for 15 D had the lowest levels of corticosterone and T3 ($P < 0.05$). In general, the levels of T4 decreased with storage duration ($P < 0.05$). At hatch, corticosterone levels increased while T4 levels decreased with storage duration ($P < 0.05$). The concentrations of T3 were comparable for embryo from eggs stored for 3 to 11 D but were higher than that of embryo from eggs stored for 15 D ($P < 0.05$). With regard to storage × development stage interaction, only corticosterone and T4 levels were affected in embryo from eggs stored for 15 D. Indeed, concentration levels were higher at hatch compared to IP, while T4 levels were lower at hatch than at IP in embryo from egg stored for 15 D ($P < 0.05$).

DISCUSSION

Storage time is well known as an important factor affecting bird egg quality and consequently embryonic development, incubation duration, and post-hatch

Table 3. Effect of storage time on embryonic mortality.

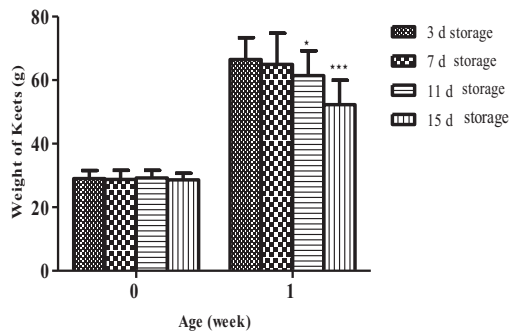
Parameters	Storage time (D)			
	3	7	11	15
Early mortality (%)	5.71 ^d	7.69 ^c	13.83 ^b	42.45 ^a
Late mortality (%)	12.54 ^d	17.70 ^c	28.36 ^b	31.80 ^a
Hatchability (%)	81.75 ^a	74.61 ^b	57.81 ^c	25.75 ^d

^{a-c}Data sharing no common letter are different ($P < 0.05$).

Table 4. Effect of storage time of incubation process.

Parameters	Storage time (D)			
	3	7	11	15
Incubation duration up to 50% IP (h)	598.36 ± 0.41 ^d	608.96 ± 0.26 ^c	617.38 ± 0.10 ^b	638.33 ± 1.04 ^a
Incubation duration up to 50% EP (h)	602.84 ± 0.28 ^d	620.13 ± 0.44 ^c	623.32 ± 0.25 ^b	645.13 ± 0.73 ^a
Incubation duration up to 50% H (h)	619.34 ± 0.36 ^d	631.66 ± 0.34 ^c	637.35 ± 0.66 ^b	661.2 ± 1.65 ^a
Duration EP-IP (h)	11.65 ± 0.40 ^c	15.09 ± 0.63 ^b	15.25 ± 0.65 ^b	19.13 ± 1.21 ^a
Duration H-EP (h)	13.67 ± 0.79 ^d	16.58 ± 0.56 ^b	17.21 ± 0.84 ^b	23.2 ± 2.27 ^a
Duration H-IP (h)	25.33 ± 6.35 ^c	31.68 ± 8.67 ^b	32.46 ± 10.44 ^b	42.33 ± 16.72 ^a

^{a-d}Means ± SD within columns with no common superscripts differ significantly ($P < 0.01$). IP = internal pipping; EP = external pipping; H = hatching

**Figure 3.** Effect of storage duration on weight of juvenile guinea fowls.

growth (Decuypere and Bruggeman, 2007). In the present study, it is pointed out that long storage time of guinea fowl hatching eggs is detrimental for embryonic parameters and post-hatch juvenile growth.

Albumen pH increased with storage duration but decreased between setting and day 6 of incubation. It is well known that storage conditions such as duration, temperature, and gaseous environment lead to increasing albumen pH and therefore affect negatively egg internal quality (Goodrum et al., 1989; Meijerhof et al., 1994; Scott and Silversides, 2000, and Tona et al., 2001). The increase of albumen pH may depend predominantly on the buffering capacity of the albumen (Benton and Brake, 1996). The effect of egg storage and its consequent rise in albumen pH on embryo survival, hatchability, and chick quality is still not fully understood. Benton and Brake (1996) suggested that the low pH of fresh eggs may be detrimental to embryo survival

and hatchability, whereas Reis et al. (1997) found no effect of pre-incubation storage on viability and hatchability in young breeders and viability was even higher in fresh eggs from older hens. However, this study shows clearly the detrimental effect of increasing albumen pH from 9.6 to 10.2 during storage on incubation duration, hatching events, and hatchability, confirming an optimum value of pH for efficient embryo development and optimum hatching performance (Reijrink et al., 2010).

The decrease in albumen pH during the first 6 D of incubation indicates its role in embryo initiation. During early incubation stage, albumen pH may influence embryo viability, but embryo viability may in turn, affect albumen pH. Reijrink et al. (2008) hypothesized that an embryo in which the hypoblast is completely formed is better able to provide an effective barrier between the internal embryo and the exterior (yolk and albumen) and/or is better able to produce sufficient amount of carbon dioxide, which will reduce the pH level in the micro environment of the embryo to the optimal pH. Surprisingly, although albumen pH increased with storage duration, there was no storage effect on albumen pH at day 6 of incubation indicating that embryo initiation may not be influenced by albumen pH at setting. However, the delay in hatching has been related to a delay in the initiation of embryogenesis (Becker et al., 1968; MacLaury and Insko, 1968; Mather and Laughlin, 1976) and in a decrease in rate of embryo development after storage. Moreover, although at day 6 of incubation albumen pH was similar between storage treatments, albumen utilization by the embryos did not followed

Table 5. Levels of corticosterone, T3, and T4 at IP and hatch according to storage durations.

Parameters	Storage time (D)			
	3	7	11	15
Corticosterone (ng/mL)				
IP	67.68 ± 8.58 ^a	67.27 ± 10.23 ^a	70.49 ± 4.92 ^a	22.78 ± 12.88 ^{b*}
Hatch	56.91 ± 13.88 ^b	63.77 ± 9.74 ^b	71.94 ± 8.09 ^b	95.39 ± 46.70 ^a
Tri-iodothyronine (T3) (pmol/L)				
IP	9.74 ± 1.01 ^a	8.69 ± 1.79 ^a	8.70 ± 3.11 ^a	5.56 ± 1.89 ^b
Hatch	8.13 ± 3.71 ^a	8.07 ± 2.00 ^a	9.17 ± 3.37 ^a	4.20 ± 1.08 ^b
Thyroxine (T4) (pmol/L)				
IP	66.58 ± 18.30 ^a	44.37 ± 4.72 ^a	37.00 ± 6.76 ^{b*}	16.98 ± 4.01 ^{b*}
Hatch	54.87 ± 10.28 ^a	38.13 ± 14.99 ^a	16.64 ± 1.42 ^b	4.17 ± 1.15 ^c

^{a-c,*}Data sharing no common letter are different ($P < 0.05$).

the same trend. From day 19 of incubation, embryos of 3 D storage consumed more rapidly albumen than those of longer storage treatments. In addition, this negative effect of storage duration on albumen utilization leads to low growth rate of the embryo of eggs stored for 7 D and more. This positive correlation between the use of albumen during incubation and the embryo growth may be explained partly by the weakness of the embryo from long storage eggs due to decreasing internal quality of those eggs. Indeed, Tona et al. (2003) reported the negative effect of long storage duration of hatching on egg internal quality, chick quality, and post-hatch growth performance of broiler chickens. It was reported that, during chicken embryo incubation, albumen is surrounded in an albumen sac by the chorio-allantois, and its proteins move into the amniotic fluid. These proteins are consumed by the embryo and then digested or transferred into the yolk sac where they can be used after hatching (Deeming, 1989). Also, during incubation, an inverse relationship between embryo weight and residual albumen was reported (Deeming, 1989; Peebles et al., 2000; and Tona et al., 2005). In addition, although embryo weights were adversely affected by storage of 7 D onwards, 1-day-old keet weights were similar between hatching egg storage treatment indicating a catch up growth during prolonged incubation time. This prolonged incubation time as well as extended duration of hatching events due to storage duration might also be explained by changes in T3, T4, and corticosterone levels at IP and at hatch. It is assumed that T3 is the calorigenically active form of thyroid and that alterations in plasma T3 would affect heat production that is necessary for hatching process. At IP, T3 and corticosterone levels were the lowest in embryo from eggs stored for 15 D compared to other storage treatments suggesting that long storage of guinea fowl hatching eggs leads to embryo weakness and consequently retarded embryonic growth and prolonged incubation time. Because the rate of T4 conversion to T3 may provide energy to the embryo, prolonged incubation time of eggs stored for 7 and 11 D compared to those stored for 3 D may be partly explained by decreasing levels of T4 with storage duration. This relationship between hormones levels and storage duration at a presumed similar developmental stage indicates that embryo weakness increases as egg storage duration increases as reported by Tona et al. (2003). Also, it was reported that corticosterone is required for the peripheral conversion of T4 to T3 during prenatal life (De Groef et al., 2008; Tong et al., 2013). Thus, the lower corticosterone level in long storage eggs might have served to reduce the shift of T4 to T3. Interestingly, the trend of changes in T3 and T4 levels in relation to storage duration were similar at IP and at hatch while corticosterone levels decreased at IP but increased at hatch with storage duration suggesting that the capability of embryos to produce corticosterone at IP leading to longer hatching events and incubation time. In the same line, Watanabe et al. (2017) showed that in ovo administration of

Corticotropin-releasing hormone in egg air chamber at day 18 of incubation shortened significantly the incubation duration.

Although 1-day-old keet weights were not affected by egg storage durations, 7 D post-hatch keet weights decreased with egg storage duration. The negative effect of increasing storage time on juvenile growth is in accordance with the report of Tona et al. (2003). These effects of storage may be due the deterioration of the egg internal quality, especially albumen height during storage (Hurnik et al., 1978; Lapaô et al., 1999; Tona et al., 2002). In addition, increasing corticosterone and decreasing T4 levels in 1-day-old keets with increasing storage duration may be an indication for stress and, therefore, result in subsequent weakness of the keets and reduced juvenile growth speed.

It can be concluded that long storage duration of guinea fowl hatching eggs increases incubation duration and consequently affects adversely hatching performance. Furthermore, long storage of eggs is detrimental for juvenile growth. Further studies are needed to establish the effect of storage on internal constituents of guinea fowl hatching eggs related to embryo development and post-hatch potential growth performance. In practice, it is recommended not to store guinea fowl hatching eggs at a temperature of 18°C more than 7 D.

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